

# Lipid Lather Removes Metals

Five of the top 20 hazardous substances on the 1999 Agency for Toxic Substances and Disease Registry/U.S. Environmental Protection Agency Priority List of Hazardous Substances are metals: arsenic, lead, mercury, cadmium, and chromium. Studies of U.S. hazardous waste remediation sites show that heavy metals are the single most prevalent class of contaminant. Metal wastes are produced by a variety of sources including mines, tanneries, and electroplating facilities, and through the manufacture of paint, metal pipe, batteries, and munitions. Metal contamination has been linked to birth defects, cancer, skin lesions, retardation, learning disabilities, liver and kidney damage, and a host of other maladies. It's estimated that the United States will spend some \$7 trillion over the next five years to clean up sites contaminated with metals.

The problem of metal contamination is not confined to the United States. Metal contamination is a global problem and can be particularly devastating in developing countries. For example, across the border in Mexico, the use of lead in gasoline (until a fairly recent ban) has contributed to seriously elevated blood lead concentrations, particularly among children. And metal wastes from industrial areas along the U.S.–Mexico border are often not disposed of properly and pose serious contamination problems in these areas. The challenge on both sides of the border is to develop methods for removing metals from soil and water. Research conducted by Raina Maier, a professor of environmental microbiology at the University of Arizona at Tucson, in collaboration with Mexican scientists including Gloria Soberon-Chavez, a microbiologist from the Instituto de Biotecnología at the Universidad Nacional Autónoma de México in Cuernavaca, offers a new solution to the problem of metal contamination of soil—using lipids to wash soil clean.

## An Alternative to Suck, Muck, and Truck

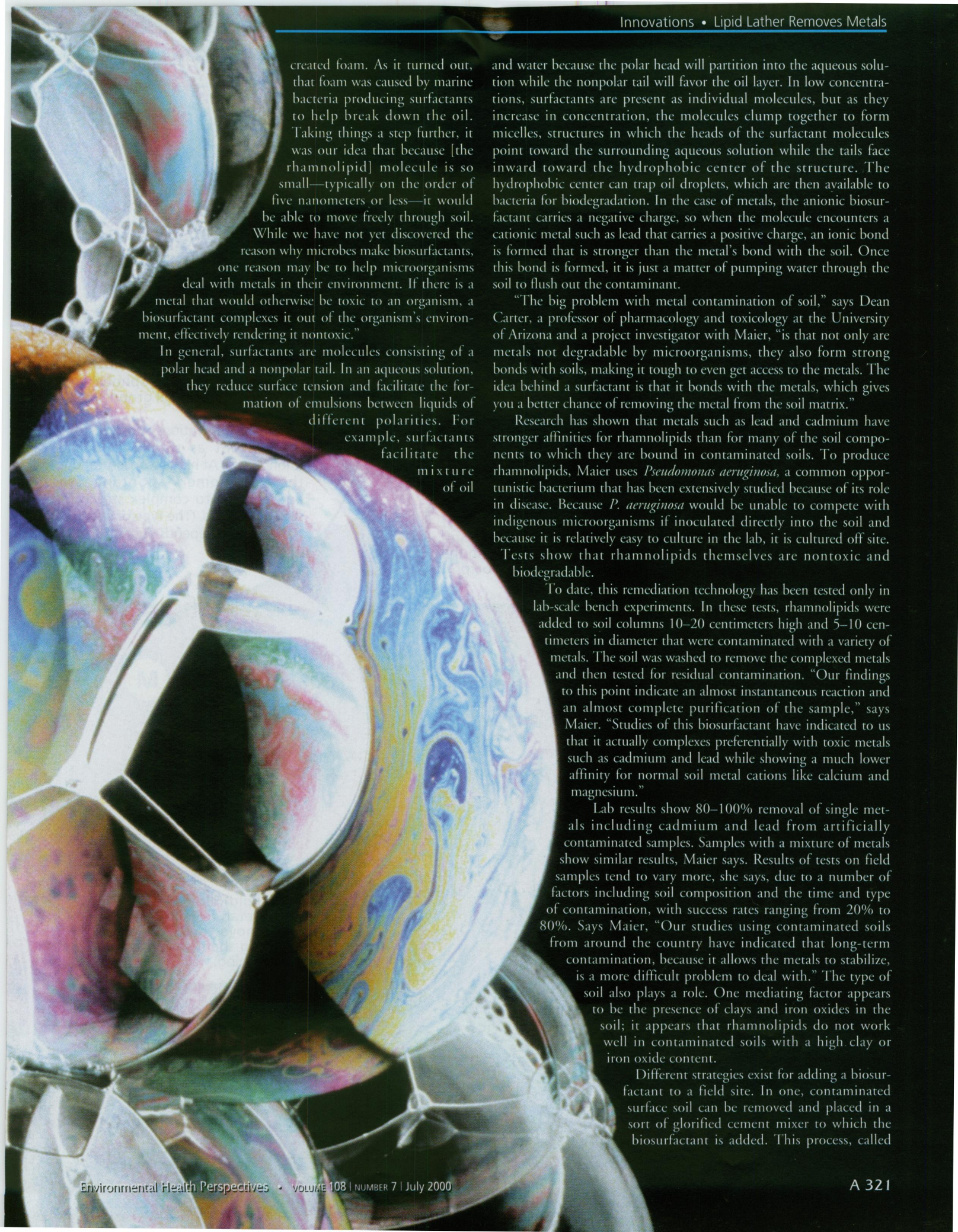
Until recently, there have been only a few methods for dealing with metal contamination in soils. One method involves digging up soil and treating it with highly concentrated acids such as nitric or hydrochloric acid to oxidize the metals in the soil. Another method—commonly referred to as “suck, muck, and truck”—consists of the time-consuming and expensive process of removing the contaminated soil and trucking it to a certified landfill. The problems with this method include cost (digging a one-acre site down to a depth of 20 inches can cost upward of \$400,000) and the fact that supposedly secure landfills are frequently rendered less so by either human error or geologic activity or a combination of the two. A third option is to use metal chelators such as EDTA (ethylenediaminetetraacetic acid), which bind to metals in soil. But EDTA, although effective, is not only nonbiodegradable but also highly toxic.

The solution proposed by Maier and colleagues uses environmentally benign biosurfactants. Biosurfactants are produced by many organisms and function similarly to the synthetic surfactants used in detergents. Just as a detergent removes grease from fabric through a binding affinity between the grease and the detergent, so do some surfactants help release organic and metal contaminants from soils.

Biosurfactants can be classified into several groups: glycolipids, lipopeptides, lipopolysaccharides, phospholipids, and fatty acids/neutral lipids. The largest and best-studied group is the glycolipid group, which includes a form known as rhamnolipids, upon which Maier's process is based. “Biosurfactants were first reported in the scientific literature perhaps 50 years ago,” says Maier. “There were reports of ocean oil spills where wave action







created foam. As it turned out, that foam was caused by marine bacteria producing surfactants to help break down the oil. Taking things a step further, it was our idea that because [the rhamnolipid] molecule is so small—typically on the order of five nanometers or less—it would be able to move freely through soil. While we have not yet discovered the reason why microbes make biosurfactants, one reason may be to help microorganisms deal with metals in their environment. If there is a metal that would otherwise be toxic to an organism, a biosurfactant complexes it out of the organism's environment, effectively rendering it nontoxic."

In general, surfactants are molecules consisting of a polar head and a nonpolar tail. In an aqueous solution, they reduce surface tension and facilitate the formation of emulsions between liquids of different polarities. For example, surfactants facilitate the mixture of oil

and water because the polar head will partition into the aqueous solution while the nonpolar tail will favor the oil layer. In low concentrations, surfactants are present as individual molecules, but as they increase in concentration, the molecules clump together to form micelles, structures in which the heads of the surfactant molecules point toward the surrounding aqueous solution while the tails face inward toward the hydrophobic center of the structure. The hydrophobic center can trap oil droplets, which are then available to bacteria for biodegradation. In the case of metals, the anionic biosurfactant carries a negative charge, so when the molecule encounters a cationic metal such as lead that carries a positive charge, an ionic bond is formed that is stronger than the metal's bond with the soil. Once this bond is formed, it is just a matter of pumping water through the soil to flush out the contaminant.

"The big problem with metal contamination of soil," says Dean Carter, a professor of pharmacology and toxicology at the University of Arizona and a project investigator with Maier, "is that not only are metals not degradable by microorganisms, they also form strong bonds with soils, making it tough to even get access to the metals. The idea behind a surfactant is that it bonds with the metals, which gives you a better chance of removing the metal from the soil matrix."

Research has shown that metals such as lead and cadmium have stronger affinities for rhamnolipids than for many of the soil components to which they are bound in contaminated soils. To produce rhamnolipids, Maier uses *Pseudomonas aeruginosa*, a common opportunistic bacterium that has been extensively studied because of its role in disease. Because *P. aeruginosa* would be unable to compete with indigenous microorganisms if inoculated directly into the soil and because it is relatively easy to culture in the lab, it is cultured off site.

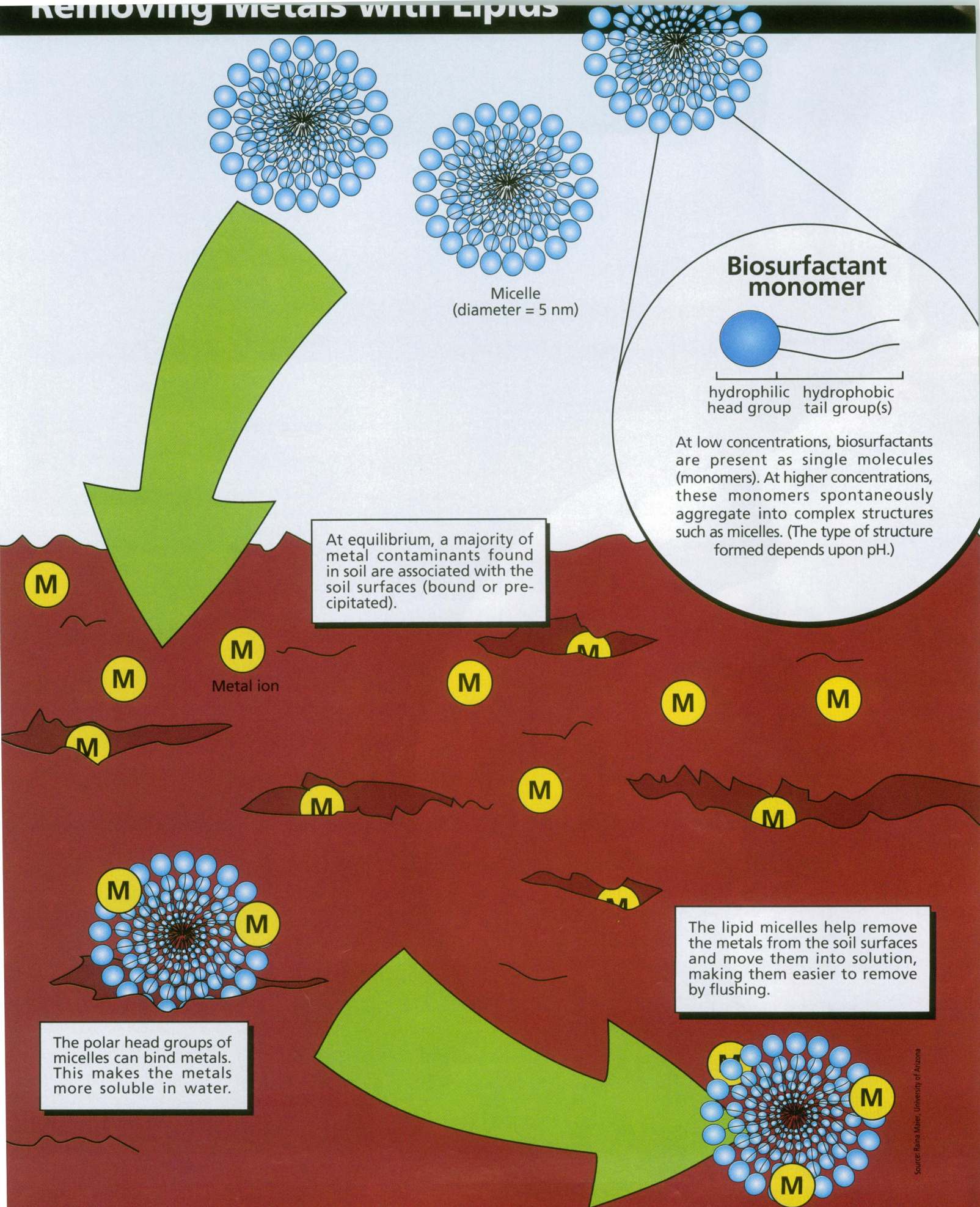
Tests show that rhamnolipids themselves are nontoxic and biodegradable.

To date, this remediation technology has been tested only in lab-scale bench experiments. In these tests, rhamnolipids were added to soil columns 10–20 centimeters high and 5–10 centimeters in diameter that were contaminated with a variety of metals. The soil was washed to remove the complexed metals and then tested for residual contamination. "Our findings to this point indicate an almost instantaneous reaction and an almost complete purification of the sample," says Maier. "Studies of this biosurfactant have indicated to us that it actually complexes preferentially with toxic metals such as cadmium and lead while showing a much lower affinity for normal soil metal cations like calcium and magnesium."

Lab results show 80–100% removal of single metals including cadmium and lead from artificially contaminated samples. Samples with a mixture of metals show similar results, Maier says. Results of tests on field samples tend to vary more, she says, due to a number of factors including soil composition and the time and type of contamination, with success rates ranging from 20% to 80%. Says Maier, "Our studies using contaminated soils from around the country have indicated that long-term contamination, because it allows the metals to stabilize, is a more difficult problem to deal with." The type of soil also plays a role. One mediating factor appears to be the presence of clays and iron oxides in the soil; it appears that rhamnolipids do not work well in contaminated soils with a high clay or iron oxide content.

Different strategies exist for adding a biosurfactant to a field site. In one, contaminated surface soil can be removed and placed in a sort of glorified cement mixer to which the biosurfactant is added. This process, called







soil washing, produces a solution containing soil and the complexed metal. After mixing, the soil is allowed to settle and the liquid solution is pumped out of the mixture, leaving clean soil. An advantage of this method, says Maier, is that the biosurfactant can be recycled and reused. One way to do this is to acidify the solution to a pH of approximately 2 to precipitate out the biosurfactant, which then can be reused. Another is to blow air through the solution to cause the biosurfactant-metal complex to begin foaming. The foam can then be skimmed off the solution surface and treated to separate the biosurfactant and metal.

Deeper, subsurface contamination requires a different process, says Maier. "Then you have to do what's called 'pump and treat,' which involves pumping the biosurfactant through the contaminated soil, pumping the contaminant-containing solution back to the surface, and then treating it to remove the metals," she says. This is a promising technology, Maier says, but she also admits it is not an all-purpose solution.

### Biosurfactant Boom on the Horizon

Biosurfactant metal remediation may still be several years from commercial use, but Maier says interest has already been expressed by several remediation and manufacturing firms, including Bio-Ohio, based in Scottsdale, Arizona, which has been in discussions involving funding research to use this technology to remove metals from sludge. Says Maier, "Here in Arizona, we have a lot of wastewater sludge that's high in copper because copper mining is a big industry in this state. Wastewater sludge makes a great soil additive, and if you could remove the excess copper and other metals, you could apply it much more freely. We're starting a project to address that possibility this summer."

Bio-Ohio representative Logan Fanjoy says his firm is developing a process to combine Maier's biosurfactant technology with another technology in development to create a whole new approach to biosolids treatment, an approach that is commercially very attractive. Says Fanjoy, "I can say

we're targeting a flow of 120 million gallons a day, with a treatment cost of less than \$10 per gallon. When you compare that to current situations, where it can cost \$90 per wet ton just to transport the biosolids, and when you figure we're looking at a treatment process that could [shorten the remediation time from] the current 18–30 days into 2 days, you have a very appealing prospect."

There are still a few hurdles to overcome before biosurfactants become a truly viable commercial alternative in environmental remediation. First would be cost, which will drop as the market builds and as improvements in the fermentation and purification processes take place. A second hurdle will be in convincing companies to use the new biosurfactant technology. Many companies have been using synthetic surfactants for a long time (estimates put the synthetic surfactant industry at sales of better than \$8 billion annually) and would need convincing to make a substantial change, especially if it involves an increase in cost.

Robert Procopio, a representative of Jeneil Biosurfactant Company, a Milwaukee, Wisconsin-based firm that makes commercial biosurfactants, says his firm is capable of producing biosurfactants in up to 20,000-gallon batches, but because the market is still developing, operates instead on a "batch to order" basis. "We use a proprietary strain of bacteria similar to the one Dr. Maier is using but which has been optimized for maximum biosurfactant production in a short period of time," he says. "It takes several weeks to make a batch, and at this time, it's more expensive than a synthetic surfactant because of the tremendous quantity in which these synthetics can be produced." However, he says, "One thing that is working in our favor is that biosurfactants are becoming competitive with synthetics on a price-performance basis. We're seeing research indicating that many formulations that might use 8–9% synthetics can perform equally well with less than 1% of a biosurfactant."

Jeneil's product is used in crude oil tank cleaning, industrial and institutional cleaning

agents, personal care products and cosmetics, environmental remediation, and other applications. The company's product has attracted a great deal of interest in environmental remediation applications from governments and private companies in Europe and Canada "primarily because of what we see as an increased sensitivity to the environmental danger posed by synthetic surfactants," Procopio says. "There are some European governments that are moving toward heavy regulation, if not the outright banning, of synthetic surfactants . . . and as that continues, the market for biosurfactants will continue to grow."

While there are still technical problems to overcome, says Soberon-Chavez, "I think this is an invaluable technology because of its effectiveness and its environmentally benign nature. I think the most difficult problem for the application of this technology is that the people involved in making decisions about how to treat specific contamination problems need to be sensitized to the benefits of this technology. I think we'll need an intensive educational campaign before we see widespread biosurfactant use in the field."

William Suk, deputy director for program development in the Division of Extramural Research and Training at the NIEHS, which provides grant funding for Maier's work, says that one factor that may speed acceptance of this technology for use along the U.S.–Mexico border, where metal contamination is a significant environmental health threat, is the growth of research collaborations between the two countries. Says Suk, "Over the past several years, we've worked more closely with Mexican academics and government officials, and I think we've succeeded in building a high level of trust. The fact that they're looking closely at the technology we've developed is a product of that growing trust. [Maier's] work is a key component of a new way of doing things that will only benefit both countries."

"I think the most important way to look at this technology is as part of a list of natural products we're only beginning to explore in any detail," says Maier. "What we've done is to show that one natural, environmentally benign product has unique metal-complexing properties. It is likely that if we explore other natural products, some with even superior properties will emerge. . . . This research should encourage us to look further to see what other biological products have these same kinds of unique properties and how we can use them to clean up contaminants in the environment."

Lance Frazer

### Suggested Reading

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